ORIGINAL ARTICLE

Variation in morphological and behavioral traits among isofemale strains of *Drosophila prolongata* (Diptera: Drosophilidae)

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Abstract

Drosophila prolongata, a member of the *rhopaloa* subgroup of the *melanogaster* species group, occurs in Southeast Asia. *Drosophila prolongata* is known to have unique and prominent sexual dimorphism, with extraordinarily thick and elongated forelegs only in males. Mating behavior of *D. prolongata* is also characteristic: males perform "leg vibration" in their courtship toward females, in which the elongated forelegs play an important role. Comparisons with closely related species suggest that these morphological and behavioral traits have evolved rapidly after the divergence of *D. prolongata*. In the present study, variation in morphological and behavioral traits was examined among *D. prolongata* strains derived from single females collected in their natural habitats. Significant variations were detected in the size of various body parts, aggressiveness of interactions between males, and mating behavior. However, no obvious relationship was observed between morphological and behavioral traits. These results suggested that genetic factors contribute to the variation in morphological and behavioral traits in *D. prolongata*. The strains characterized in this study are useful for studies on the genetic mechanisms underlying the evolution of characteristic traits in *D. prolongata*.

Key words: aggressive interactions, mating behavior, polymorphism, sexual dimorphism.

INTRODUCTION

In insects, morphological and behavioral traits are determined by genetic factors as well as environmental conditions. Thereby, even within the same species, genetic variability in morphological and behavioral traits is observed not only among different geographic populations but also within a population (David *et al.* 2005, 2006; Narraway *et al.* 2010; Pavkovic-Lucic & Kekic 2011; Stange & Ronacher 2012). Such variation within a population is sometimes stably maintained as alternative ecological strategies (Cabral *et al.* 2008). For example, in the mimetic butterfly *Heliconius numata*

(Cramer, 1780), several discrete wing color patterns are maintained in sympatry, which are under the genetic control of a single supergene locus (Joron *et al.* 2006, 2011; Brower 2013). In the adzuki bean beetle *Callosobruchus chinensis* (Linnaeus, 1758) and the red flour beetle *Tribolium castaneum* (Herbst, 1797), duration of death-feigning behavior was variable within a population. Strains showing extremely long death feigning, as well as those with almost no death feigning, were established by successive artificial selection, suggesting that the variation in death-feigning behavior is determined genetically and maintained within a population as alternative anti-predator strategies (Ohno & Miyatake 2007; Miyatake *et al.* 2008; Nakayama & Miyatake 2009). In the common fruit fly *Drosophila melanogaster* Meigen, 1830, genetic variability in aggressive behavior was found among strains derived from a wild population (Edwards *et al.* 2009; Zwarts *et al.* 2012). Because genetically determined variation in phenotypic traits is the subject of natural selection in

Darwinian evolution, studies on genetic variability in current populations provide insight into the evolutionary mechanisms that have formed the characteristics observed in the current species (David *et al.* 2005; Pigliucci 2008).

Drosophila prolongata Singh & Gupta, 1977, a member of the *rhopaloa* subgroup of the *melanogaster* species group, is distributed in southwestern China, northeastern India, Myanmar and Vietnam (Singh & Gupta 1977; Toda 1991; Setoguchi *et al.* 2014). Unlike other drosophilids, the body size of *D. prolongata* is larger in males than females, and the forelegs are enlarged only in males. The enlarged forelegs are used in aggressive interactions between males, as well as in a specific courtship behavior, "leg vibration", in which the male vigorously vibrates the female's abdomen by extending his forelegs from in front of her (Setoguchi *et al.* 2014). These morphological and behavioral characteristics are not observed in closely related species (*Drosophila kurseongensis* Gupta & Singh, 1978, *Drosophila rhopaloa* Bock & Wheeler, 1972, and undescribed species KB866), suggesting that these traits have evolved rapidly after the divergence of *D. prolongata* (Setoguchi *et al.* 2014). Because the large forelegs are necessary to accomplish leg vibration, the morphology and the behavior appear to be functionally linked to each other. Leg vibration was often observed before copulation attempts, suggesting that it has an adaptive role in the mating process. Nevertheless, leg vibration is not an indispensable element of courtship behavior, because one-half of successful copulations were not preceded by leg vibration (Setoguchi *et al.* 2014).

In the present study, genetic variability in morphological and behavioral traits in *D. prolongata* was examined using 15 isofemale strains derived from natural populations. Significant variation among strains was found in the sizes of various parts of the adult body, aggressiveness of interactions between males, and mating behavior. These results provide basic information for studying the genetic mechanisms underlying the evolution of characteristic traits in *D. prolongata*.

MATERIALS AND METHODS

Drosophila prolongata strains

Strains used in this study were directly established from single mated females collected at two sites (Sa Pa, 22°20′N, 103°52′E; Ba Vi, 21°04′N, 105°22′E), Vietnam. SaPa001, SaPa002, SaPa004, SaPa006, SaPa008, SaPa010, SaPa012 and SaPa014 were collected by H. Takamori in September 2004. BaVi042, BaVi043 and BaVi044 were collected by H. Takamori in

March 2005. SaPa-A, SaPa-B, SaPa-C and SaPa-D were collected by H. Watabe in September 2010. The established isofemale strains were maintained on ordinary cornmeal medium for *Drosophila* culture.

Measurement of body size

To avoid the effect of overcrowding, larvae were reared at a controlled density. Twenty newly hatched larvae were collected within 24 h after egg laying, and transferred into a vial (25 mm in diameter × 100 mm in height; Asahi Glass Co., Tokyo, Japan) containing 8 mL of the cornmeal medium and maintained at 20°C under conditions of 12 h light : 12 h dark (LD 12:12). Three replicates were made for each isofemale strain. After eclosion of adults, five males and females each were collected from the respective vials. In total, 15 males and females were used for the measurement of body size for each strain. Images were acquired using a digital camera (IUC-300CN2, Trinity Inc., Saitama, Japan) mounted on a dissecting microscope (Olympus Co., Tokyo, Japan). The sizes of body parts were measured using Image J software v1.47 (Research Service Branch, National Institute of Mental Health, MD, USA). Measurements were made for 12 aspects of the head, thorax, wing, foreleg, and midleg (Fig. 1). Pictures were taken from the dorsal side of the head, and from the lateral side of the thorax. Left wings and legs were dissected and mounted on a slide glass before acquisition of images.

Behavior analysis

Newly eclosed males were maintained individually in a vial (25 mm in diameter × 100 mm in height, containing the cornmeal medium) for six days, and then individually starved for one day in an empty vial with wet paper. Females were prepared in the same way but in groups. A glass chamber (50 mm in diameter × 70 mm in height; Nichiden-Rika Glass Co., Kobe, Japan) the wall of which was treated with silicon polish to prevent flies from climbing, was used for observation of the behavior of flies (Fig. 2). A disc of wet filter paper was placed on the bottom of the chamber, and Drosophila instant medium (Formula 4–24 Drosophila Medium, Carolina Biological Supply Co., NC, USA) filled in the lid of a 15 mL conical tube (23 mm in diameter × 11 mm in height) was placed on it (Fig. 2). Under these conditions, humidity in the chamber was maintained at approximately 80% (measured using Hygropalm HP22-A thermo-hygrometer with an HC2-P05 probe, Rotronic, Bassersdorf, Switzerland). Four observation chambers, which were isolated from each other by cardboard partitions (35 mm in height), were covered with a glass plate. A digital camera (HDR-CX720V; Sony, Tokyo,
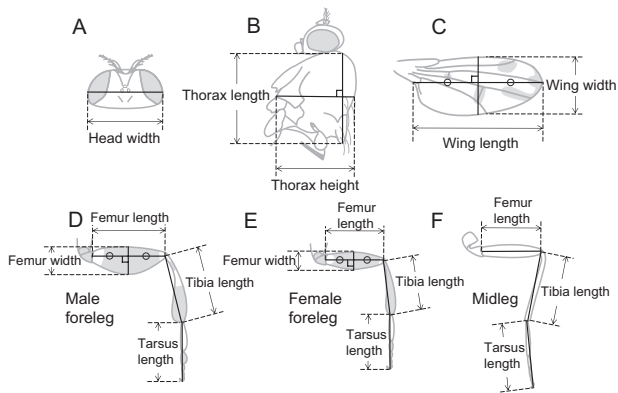
Figure 1 Measurement of body size. (A) Head width: length of a segment of a line passing through the base of the ocellar triangle connecting the head edges. (B) Thorax height: length of a segment of a line connecting the edge of the mesonotum and the bottom of the katepisternum passing through the base of the anterior dorsocentral seta; thorax length: length of a segment of a line perpendicular to the line drawn for "thorax height", and connecting the tip of the scutellum and the edge of the mesonotum. (C) Wing length: length of a segment of a line connecting the base of the anterior cubital of alula and the end of the third longitudinal vein; wing width: length of the segment of a perpendicular bisector of the "wing length" connecting the wing edges. (D–F) Foreleg and midleg femur length: length of a segment of a line connecting the center of the boundary of the trochanter and the center of the boundary of the tibia; foreleg femur width: length of a segment of a perpendicular bisector of the "foreleg femur length" connecting the foreleg edges; foreleg and midleg tibia length: length of a segment of a line connecting the center of the boundary of the femur and the center of the boundary of the tarsus; foreleg and midleg tarsus length: length of a segment of a line connecting the center of the boundary of the tibia and the tip of the tarsus.
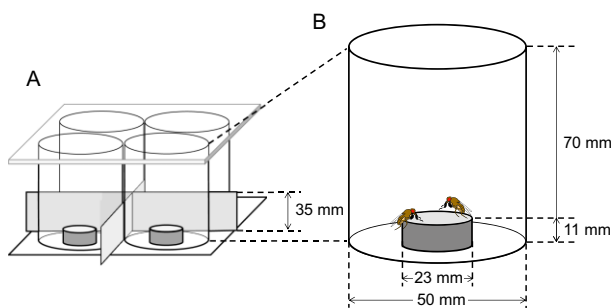
Figure 2 Setup for behavior analysis. (A) Behaviors of the flies in four chambers were video-recorded simultaneously. Visual interaction between flies in neighboring chambers was prevented by cardboard partitions. (B) Close-up of a chamber. Food was provided at the center of the chamber as an arena for aggressive and mating interactions. See text for detailed description.

Japan) was installed 80 cm above the chamber. Recordings started at 9.5 h after the start of the light phase (in our preliminary observation, fighting and mating activity had two peaks in the morning and evening; A. Kudo,

unpubl. obs., 2013). Two males from the same strain were introduced into a chamber and their aggressive interaction was recorded for 1 h. Then, a female from the same strain was introduced into the chamber, and mating behavior was recorded for an additional 1 h. Recordings were played back on a personal computer and inspected visually. Two males in a chamber were not distinguished in the scoring of aggressive interaction and mating behavior. For each strain, at least 30 replicates were made.

Statistics

Morphological traits were analyzed by MANOVA with Wilks' lambda. Because the interaction between sex and strain was significant, males and females were separated in the following principal component analysis (PCA) on standardized data. The significance of differences between strains was examined by pairwise *t*-test for morphological traits. For behavioral traits, the significance of differences between strains was examined by pairwise Wilcoxon rank sum test following Kruskal–Wallis rank sum test, except for the count data that were examined by either the G-test or Fisher's exact test and the data of latency to copulation was examined by the Gehan–Wilcoxon test. The relationship between morphological and behavioral traits was examined by principal component regression using the mean values for each strain. Three morphological datasets (male sizes, female sizes, and male/female ratios; Appendix S1) were independently analyzed to explain each behavioral trait (Appendix S4). The *P*-values in multiple comparisons were adjusted by the Bonferroni method. All analyses were performed using the R v2.15.3 (R Core Team 2013).

RESULTS

Variation in morphological traits

Morphological data are shown in Table 1. As expected, the difference between sexes was largest for the forelegs. Although it was not prominent as forelegs, midlegs were also sexually dimorphic. The size ratio between sexes was 1.5–2.8 for the forelegs, 1.3–1.5 for the midlegs, but only 1.1 for the head, thorax and wing (Table 1). The effects of sex and strain, and the interaction between them were significant by MANOVA (Table 2), suggesting that the degree of sexual dimorphism was different between strains. Therefore, males and females were analyzed separately in the following PCA (Table 1, Fig. 3, Appendices S2,S3). Among the twelve principal components (PCs), PC1 explained about one-half of the variance both in males and females (Appendices S2,S3). Because all of the loadings had minus values, PC1 may

Table 1 Summary of morphological analyses

Body part	Mean ± SD (mm)			PC1[†]		PC2[†]	
	Male	Female	M/F	Male	Female	Male	Female
Head width	1.21 ± 0.04	1.12 ± 0.03	1.08	−0.79	−0.75	0.18	0.31
Wing width	1.65 ± 0.05	1.44 ± 0.04	1.12	−0.69	−0.77	0.26	0.32
Wing length	1.68 ± 0.09	1.50 ± 0.09	1.11	−0.81	−0.87	0.08	0.02
Thorax height	1.48 ± 0.06	1.32 ± 0.05	1.15	−0.70	−0.74	0.40	0.28
Thorax length	3.17 ± 0.05	2.85 ± 0.05	1.12	−0.61	−0.81	0.40	0.28
Foreleg femur width	0.57 ± 0.03	0.20 ± 0.02	2.85	−0.56	−0.23	−0.04	0.54
Foreleg femur length	1.54 ± 0.06	0.84 ± 0.03	1.83	−0.73	−0.83	−0.17	−0.21
Foreleg tibia length	1.43 ± 0.06	0.76 ± 0.05	1.88	−0.81	−0.75	−0.07	−0.20
Foreleg tarsus length	1.19 ± 0.06	0.80 ± 0.04	1.49	−0.44	−0.61	−0.70	−0.53
Midleg femur length	1.25 ± 0.05	0.93 ± 0.04	1.34	−0.78	−0.72	0.04	−0.01
Midleg tibia length	1.35 ± 0.06	0.97 ± 0.04	1.39	−0.81	−0.80	−0.07	−0.15
Midleg tarsus length	1.39 ± 0.06	0.94 ± 0.04	1.48	−0.59	−0.60	−0.61	−0.47

[†]Factor loadings from the principal component analysis. Males and females were separately analyzed (see text). M/F, male/female ratio.
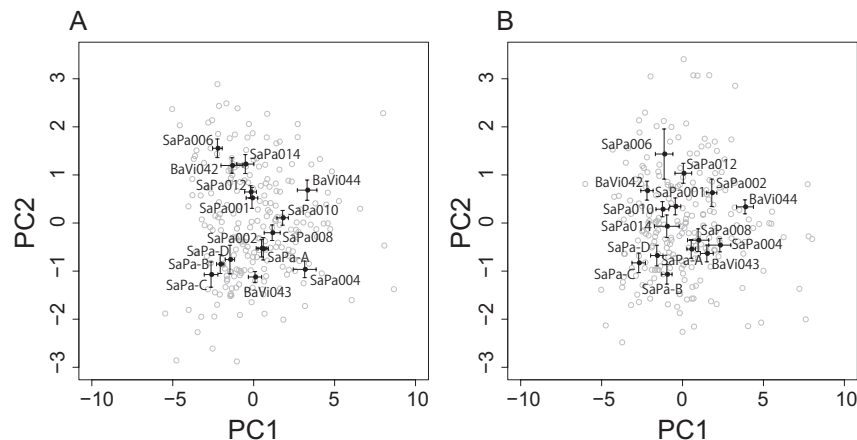
Figure 3 Scatter plot of PC1 and PC2 scores. Open circles represent individuals. Filled circles and error bars represent mean and standard error of each strain, respectively. PC1 represents body size (the larger the value of PC1, the smaller the body size), and PC2 represents leg proportion to body size (the larger the value of PC2, the smaller the leg-to-body proportion). (A) Males. (B) Females. PCs were derived from independent PCA for males and females; thus the scores for males and females should not be compared directly. Nevertheless, the loadings of PC1 and PC2 had similar tendencies between males and females (Table 1).

Table 2 Results of MANOVA for the effects of sex and strain and their interaction on morphological traits

Effect	df	Wilks' lambda	F	P
Sex	1	0.006	5845.6	<0.001
Strain	14	0.019	9.2	<0.001
Sex × strain	14	0.299	3.0	<0.001

represent general body size; the larger the body size, the smaller PC1 became. The contribution of the other PCs was much smaller compared with that of PC1. PC2 was loaded positively by the head, thorax and wing, whereas it was loaded negatively by the foreleg and midleg, suggesting that PC2 represents the proportion of leg size to

other body parts. It should be noted that PC2 was heavily loaded by the length of tarsi, whose size variation was not obvious by visual inspection. The other PCs were difficult to explain in a biological context.

Variation in behavioral traits

Aggressive behavior

Aggressive behavior in *Drosophila* consists of multiple behavioral components (Zwarts *et al.* 2012). Among them, boxing is classified as an offensive interaction of the highest intensity level, in which both flies rear up on hind legs and strike the opponent with their forelegs. The total duration of boxing was significantly different between strains (Table 3), suggesting that variation in

Table 3 Behavioral characteristics of each strain

| Strain	*n*	Boxing duration[†] (min)	Number of copulations	Number of leg vibration occurrences	Leg vibration immediately before first copulation
BaVi042	31	0.65 ± 0.3 ade[‡]	0.29 ± 0.1 c[§]	0.90 ± 0.6 bd[§]	0.56 ab[¶]
BaVi043	32	3.00 ± 0.8 abde	0.91 ± 0.1 a	1.03 ± 0.2 ab	0.79 a
BaVi044	32	0.47 ± 0.1 acde	0.59 ± 0.1 ac	1.25 ± 0.3 abd	0.68 ab
SaPa001	31	0.60 ± 0.3 ade	0.58 ± 0.1 ac	1.06 ± 0.3 abd	0.50 ab
SaPa002	31	1.19 ± 0.2 ab	1.10 ± 0.1 ab	1.00 ± 0.3 abc	0.43 ab
SaPa004	30	2.67 ± 0.8 b	0.77 ± 0.1 abc	1.23 ± 0.2 abd	0.64 ab
SaPa006	31	0.22 ± 0.1 de	0.65 ± 0.1 abc	2.26 ± 0.4 ac	0.63 ab
SaPa008	31	8.40 ± 1.4 b	0.68 ± 0.1 abc	0.42 ± 0.1 bd	0.32 ab
SaPa010	31	0.32 ± 0.1 de	1.29 ± 0.1 ab	1.90 ± 0.7 abcd	0.45 ab
SaPa012	33	1.72 ± 0.4 bc	0.33 ± 0.1 c	4.21 ± 0.8 c	0.33 ab
SaPa014	31	0.28 ± 0.1 de	0.94 ± 0.0 ab	0.32 ± 0.1 d	0.21 b
SaPa-A	31	1.65 ± 0.4 abd	1.00 ± 0.1 ab	1.32 ± 0.2 ac	0.77 a
SaPa-B	31	1.14 ± 0.3 abde	1.06 ± 0.1 ab	1.52 ± 0.2 ac	0.47 ab
SaPa-C	30	0.39 ± 0.1 ade	0.70 ± 0.1 ac	0.67 ± 0.2 bd	0.47 ab
SaPa-D	31	0.25 ± 0.1 e	0.58 ± 0.1 ac	0.52 ± 0.1 bd	0.59 ab

[†]Sum of all bouts for each pair. [‡]Values followed by the same letter are not significantly different at $P > 0.05$ after Bonferroni correction by pairwise Wilcoxon rank sum tests. [§]Values followed by the same letter are not significantly different at $P > 0.05$ after Bonferroni correction by G-test. [¶]Values followed by the same letter are not significantly different at $P > 0.05$ after Bonferroni correction by Fisher's exact test.

aggressiveness is at least partly determined genetically in *D. prolongata*. In most cases, boxing was observed only early in the observation period, whereas low intensity interactions (leg fencing and wing threat) were observed even after the winner was determined (data not shown). Consequently, boxing duration was shorter than encounter duration (total time for which both flies were on the food surface) in most pairs (Fig. 4). However, many pairs of SaPa008 and BaVi043 spent a large part of the time boxing, sometimes exceeding the encounter duration (Fig. 4, data points above the line of $y = x$). This happened when flies continued boxing even after they fell off the food surface. These results suggested that SaPa008 and BaVi043 are hyper-aggressive strains.

Mating behavior

The number of copulations was significantly different between strains (Table 3). Mean values exceeding one indicate that multiple copulations occurred. Although the two males in a chamber were not distinguished in our observations, more than two copulations were observed in some cases (Table 3, Fig. 5), showing that the same male copulated multiple times. Despite the high copulation rate, multiple copulation was not observed in SaPa014 and BaVi043 (Fig. 5). Latency to the first copulation after the introduction of a female was 20–30 min in most strains (Appendix S4). The duration of each copulation bout was approximately 5 min in most strains (Appendix S4).

Leg vibration is a component of courtship behavior specifically observed in *D. prolongata*. The incidence of
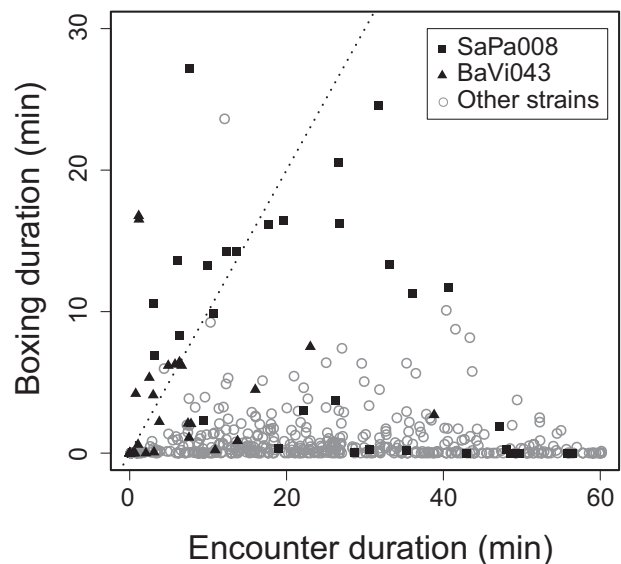
Figure 4 Plot of boxing duration against encounter duration. Encounter duration represents the total time for which both flies were on the food surface. Note that the scales of *x* and *y*-axes are different. Dotted line represents $y = x$. Each point represents a single pair. Boxing duration is usually less than the encounter duration (below the line of $y = x$). However, in SaPa008 and BaVi043, flies often continued boxing even after they fell off the food surface, resulting in longer boxing duration than encounter duration.

leg vibration differed between strains (Table 3). Although SaPa012 showed a high incidence of leg vibration, the number of copulations was low. In contrast, most pairs copulated successfully in SaPa014, in which
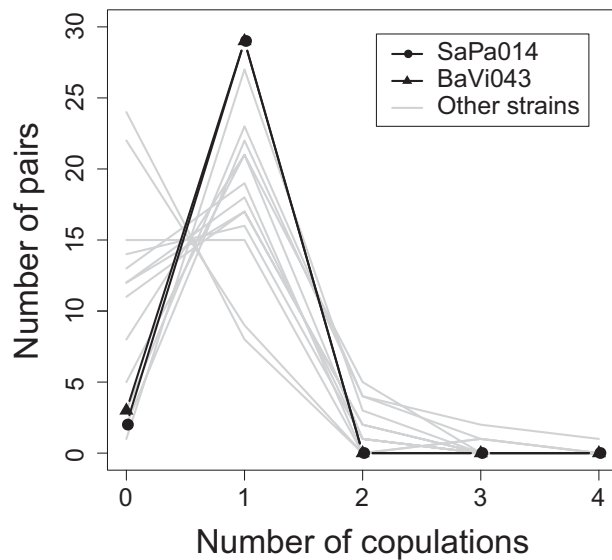
Figure 5 Distribution of the number of copulations. Each line represents a single strain. Two strains, SaPa014 and BaVi043, showed high copulation rates (=small number of pairs for 0 times of copulation), whereas multiple copulations were never observed in these strains.

the incidence of leg vibration was low. These two extreme examples suggest that the incidence of leg vibration was not positively related to copulation success, at least under the present experimental conditions. Duration of each bout of leg vibration was constant, and total duration of leg vibration roughly correlated with the number of leg vibrations.

To further characterize the effect of leg vibration on copulation success, the occurrence of leg vibration immediately before the first copulation was examined. The proportion of copulations preceded by leg vibration was significantly different between strains (Table 3). In SaPa-A and BaVi043, about 80% of first copulations were accompanied by leg vibration, whereas only 20% were accompanied by leg vibration in SaPa014. The number of copulations was not significantly different between these three strains, suggesting that leg vibration was not related to copulation success. However, it was noteworthy that SaPa014 was one of the strains that did not show multiple copulations. It remains possible that leg vibration is not indispensable for first copulation, but required for multiple copulations.

Relationship between morphological and behavioral traits

To examine if there were any relationships between morphological and behavioral traits, three morphological datasets (male sizes, female sizes and male/female ratio)

were independently analyzed to explain each behavioral trait by principal component regression. However, obtained models were not significant in all combinations (*F*-test; $P > 0.05$ after Bonferroni correction), suggesting that variations in morphological and behavioral traits observed in this study were not genetically correlated.

DISCUSSION

In this study, variations in morphological and behavioral traits were found among "isofemale" strains of *D. prolongata*, suggesting the presence of natural genetic variation in these traits. Such variations among the strains provide some insights into the prominent morphological characters and behaviors of this species, which were not found in our previous study using only one isofemale strain (Setoguchi *et al.* 2014). The analysis of behavioral traits identified several strains with impressive phenotypes. SaPa008 and BaVi043 showed extreme aggressiveness in the male-to-male interaction (Fig. 4), whereas SaPa006, SaPa014 and SaPa-D showed the least aggressiveness (Table 3). SaPa008 also showed the longest copulation duration. The number of copulations was significantly different between SaPa010 and BaVi043. SaPa010 tended to mate earlier, and showed the highest number of copulations, implying that male courtship activity and/or female receptivity was high in this strain. Large variation in behavioral traits might be derived from positively maintained variations in the natural population. Unfortunately, little is known about the ecology of *D. prolongata* in their natural habitat. For example, it is unknown on which resources they breed. We observed that males of *D. prolongata* showed territory behavior in their natural habitat (T. Matsuo, H. Takamori & H. Watabe, unpubl. obs., 2013). When small pieces of banana were placed on the ground, single males occupied the top surface of the food. In *Drosophila elegans* Bock & Wheeler, 1972 and *Drosophila gunungcola* Sultana, Kimura & Toda, 1999, males occupy and defend individual *Ipomoea* flowers as territories so as to mate with females visiting the flower (Kimura & Hirai 2001; Suwito *et al.* 2012). Males of *D. prolongata* might hold territories for mating. Interestingly, subordinate males stayed on the side of the banana pieces, hiding from the dominating male, and frequently joined in the courtship when a female appeared. This observation suggests that there may be alternative mating strategies in *D. prolongata* under the natural condition. Although there was no correlation between morphology and behavior (for example, males with longer legs did not tend to be more aggressive or perform more leg vibrations), behavioral variations observed in this study may imply the existence of

complex ecological interaction between individuals of *D. prolongata*, as discussed below.

Males are smaller than females in most drosophilids (Markow 1996, 2002; Moreteau *et al*. 2003; Yassin *et al*. 2011); for example, the size ratio of males to females in *D. melanogaster* is about 0.87–0.90 (Chakir *et al*. 2008, 2011). In contrast, males were larger than females in *D. prolongata* (Table 1). Among many models that concern sexual dimorphism in body size, three may explain the case in *D. prolongata*. First, where competition among males is frequent, larger males are likely to dominate, acquiring the opportunity to mate with more females (Fujisaki 1981; Miyatake 1993; Hack 1997; Judge & Bonanno 2008). In fact, aggressive interactions between males were prominent in *D. prolongata*. Second, females may actively choose high-quality mates with larger body sizes through detecting the conditions of specific male traits (Simmons 1986; Kotiaho *et al*. 1996). In *D. prolongata*, the loadings of PC1 showed that foreleg size was positively correlated with body size (Table 1), suggesting that foreleg size is a good indicator of whole body size. Third, in species in which females are reluctant to mate, larger males are able to subdue females more efficiently to achieve matings (Allen & Simmons 1996; Sugano *et al*. 2008; Jorge & Lomonaco 2011). In this regard, it might be worth noting that the duration of each copulation bout was longest in SaPa008, the most aggressive strain. These three possibilities remain to be examined by further studies.

Re-mating is commonly observed in females of *Drosophila* species, but it usually requires an interval of several days between first and second copulations (Pyle & Gromko 1981; Gromko & Newport 1988). However, in some strains of *D. prolongata*, females copulated repeatedly within the one-hour observation period (Table 3, Fig. 5). Re-mating causes sperm competition where the first and second males compete for paternity of the offspring (Parker 1970; Birkhead & Pizzari 2002; Fricke *et al*. 2009; Wong 2011). In this regard, it was also characteristic in *D. prolongata* that the duration of each copulation bout was one-half of that in other *Drosophila* species: 5.30 min on average in *D. prolongata*, compared with about 10 min in the majority of *Drosophila* species (Markow 1996). Immediate acceptance of re-mating by females may benefit males by compensating for the short duration of each copulation bout, whereas it may increase the risk of sperm competition with other males (Bretman *et al*. 2009; Mazzi *et al*. 2009). On the other hand, a shorter duration of copulation and frequent re-mating are generally considered to benefit females (Arnqvist & Nilsson 2000). Thus, in *D. prolongata*, ecological power balance between males and females through sperm competition may differ from that in other *Drosophila* species. Nevertheless, SaPa014 and BaVi043 copulated only once despite the high copulation rate (Table 3, Fig. 5), suggesting that females of these strains accepted copulation once and then refused re-mating, as reported in other *Drosophila* species. These strains with different re-mating tendency would be a good material for studying the ecological mechanisms underlying re-mating and sperm competition.

Variations in behavioral traits remain to be further examined from several aspects. For example, it is not clear which sex, male or female, is responsible for the observed variation in mating behavior. Behavioral strategy is known to be altered in response to morphological and behavioral traits of the counterparts (Sugano & Akimoto 2007; Fitzsimmons & Bertram 2013). Thus, aggressive interactions and mating behavior need to be examined when individuals from different strains are paired. Some illustrative coincidences of independent behavioral characteristics, for example, high aggressiveness and long duration of copulation, were observed. However, because such coincidences were observed only in a few strains, the association between these traits remains to be dissected genetically by further analysis.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1 Size difference of the morphological traits of each strain.
Appendix S2 Factor loadings from the principal component analysis of morphological traits in males.
Appendix S3 Factor loadings from the principal component analysis of morphological traits in females.
Appendix S4 Results of behavioral analyses.